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RESEARCH ARTICLE

Stability Indicating HPLC Method for Simultaneous Determination of Ketoprofen and Methylparaben in Gels

Vania Maslarska*

Department of Chemistry, Faculty of Pharmacy, Medical University – Sofia, Bulgaria.

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ABSTRACT

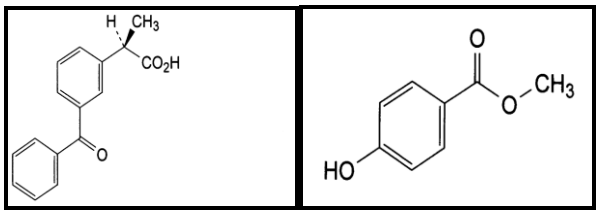
A simple and fast reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of ketoprofen – an active component and methylparaben – a preservative, presented in the 2.5 % Ketoprofen gel. The chromatographic separation was performed on a LiChrosorb C18 column; the mobile phase for separation of these compounds consisted of a mixture of 140 ml acetonitrile: methanol (60:40 v/v) and 160 ml 0.5% ammonium acetate. The pH was adjusted to 5.9 with 10 % nitric acid. The analysis time was less than 5 min, at a flow rate 2.0 ml/min and detection at 254 nm. The method was found to be applicable for routine analysis (stability tests, homogeneity) in the pharmaceutical product – Ketoprofen gel 2.5%.

KEYWORDS

Ketoprofen, Methylparaben, Preservatives, HPLC, Validation

INTRODUCTION

Ketoprofen (2-(3-benzoylphenyl)-propionic acid (KETO), is a derivative of propionic acid (Fig.1). It is a non-steroidal anti-inflammatory drug (NSAID) and analgesic agent[1]. Ketoprofen is mainly used in human therapy in the treatment of arthritis because of its analgesic and anti-inflammatory properties.



Ketoprofen Methylparaben

Figure 1: Major component and preservative product in 2.5 % Ketoprofen gel

***Address for Correspondence:**
Maslarska Vania
Faculty of Pharmacy,
Dunav 2 str., Sofia 1000, Bulgaria.
E-Mail Id: vmaslarska@mail.bg

Methylparaben (MP) is effective antibacterial and antifungal agent, which is commonly used as a preservative in foods, beverages, cosmetics and pharmaceuticals[2].

Several methods have been described for Ketoprofen and MP determination in pharmaceutical formulations including UV spectrophotometry[3,4], chromatography[5-9] and colorimetry[10,11]. In addition, there are some NMR[12], electrochemical[13] and FT-IR[14] methods used for the quantitative determination of Ketoprofen. A different study has been conducted by Blanco et al., using a derivative UV spectrophotometric method and performing the quantitative determination of Ketoprofen & paraben[15] in that they eliminate the interference problem caused by the paraben. Other authors have used SPE and UV-spectrophotometric method in order to solve the interference in gels resulted from the preservatives[16]. Nowadays, HPLC analysis has been extensively applied in routine analysis of pharmaceuticals.

The purpose of this study was to develop an HPLC method for the determination of Ketoprofen and Methylparaben in the topical gel Ketoprofen 2.5% - containing ketoprofen, carbopol, ethyl alcohol, triethanolamine, methylparaben and distilled water. This method was validated and successfully applied to the separation, quantification and stability study of these two components in the pharmaceutical formulation Ketoprofen gel 2.5%.

MATERIALS AND METHODS

Reagents and Materials

The working standard of Ketoprofen RS (Purity>99%) was provided by (Sigma-Aldrich). The pharmaceutical formulations containing ketoprofen (Ketoprofen gel 2.5%) were obtained from Vetprom Company in Bulagaria. Additives in gels were purchased from Sigma. Acetonitrile and Methanol were HPLC grade, Sharlau. All other reagents were analytical grade and all solutions were prepared with bidistilled water.

Instrumentation

The HPLC system consisted of model Shimadzu series 20AD solvent delivery system with a Shimadzu DGU-20A_5 vaccum degasser, a Shimadzu SPD-20A UV/VIS detector set to 254 nm. A LiChrosorb RP-18 column (250x4 mm i.d. 10 µm particle size) was used. A HPLC system was equipped with LC solution software for data processing. Typical operating conditions included flow rate, 2.0 ml/min; operating temperature, room temperature; injection volume, 20 µl. The run time was 5 min.

Chromatographic Conditions

The mobile phase was a 140 ml mixture of Acetonitrile: Metanol (60: 40% v/v) and 160 ml 0.5 % (w/v) solution of ammonium acetate and the pH adjusted to 5.9 by the addition of nitric acid. After mixing, the mobile phase was filtered through membrane filter 0.45 µm and degassed with ultrasonic bath for 10 min.

Preparation of Solvents and Solutions

A solvent A was prepared by mixing 275 ml acetonitrile and 550 ml 0.5% w/v solution of ammonium acetate. In order to prepare Ketoprofen stock solution, 25.0 mg KETO was accurately weighed, dissolved and diluted to 25.0 ml with methanol.

To prepare Methylparaben stock solution, 10.0 mg MP was accurately weighed, dissolved and diluted to 100.0 ml with methanol.

Five milliliters of the Ketoprofen stock solution, two milliliters of the Methylparaben stock solution and 19 ml methanol were transferred and then diluted to 100.0 ml with solvent A. The final concentration of 50 µg/ ml KETO and 2 µg/ ml MP, respectively was obtained.

Ketoprofen standard solutions ranging from 13.0 to 104 µg/ ml were prepared with the solvent A.

Methylparaben standard solutions ranging from 0.55 to 4.4 µg/ ml were prepared with solvent A.

All resulting solutions were filtered through Whatman filter paper.

Analysis of Gels

Quantity of the gel equivalent of ten milligrams Ketoprofen was accurately weighed, dissolved in 50 ml methanol and sonicated in an ultrasonic sonicator for 10 min and centrifuged at 3500 rpm for 5 min. Five milliliters of the clear supernatant was diluted to 25.0 ml with solvent A. In order to determine Ketoprofen and Methylparaben content in the gel, Ketoprofen standard solutions and Methylparaben standard solutions were injected and calibrations curves were obtained as peak areas versus concentration. Twenty microliters of gel solution was injected at wavelength of 254 nm. By using calibration curve, quantitative determination of Ketoprofen in gel was performed.

Stability Studies

Amount of gel equivalent to about 25 mg KETO was separately transferred to four different 25.0 mL volumetric flasks (Flask No. 1, 2, 3 and 4) and 5.0 mL of 0.1 mol/l HCl; 0.1 mol/l NaOH and 3% H_2O_2 were added to Flask No. 1, 2 and 3, respectively. Solutions in flask No. 1, 2, and 3

were heated in water bath for 3 h at 80°C. Flask No. 4 containing gel was kept at 60°C for 24 h to study the effect of heat on gel sample (heat degradation). The forced degradation was performed in dark to exclude the possible degradative effect of light. All gel samples were treated and analyzed in similar manner as described under analysis of gel formulation. Results of forced (stress) degradation studies are shown in Table 5.

Method Validation

Method Development & Optimization

For developing an efficient method for analysis, parameters, such as detection wavelength, mobile phase composition, optimum pH and concentration of the standard solutions were comprehensively studied. All the ingredients were diluted in dilution solvent and then run through UV spectrophotometer in UV range of 190nm - 400nm to get maximal wavelengths, where maximum absorbance was gained i.e. 260 nm and 254 nm for KETO and MP respectively. However, considering the difference of the ingredients concentration and the intensity of their absorbance a single wavelength method was adopted, that is 254 nm at which all the molecules gave a satisfactory absorbance. The chromatographic parameters were evaluated using a LiChrosorb® C18 column. The mobile phase composed of Acetonitrile: Methanol of previously mentioned proportion promoted a short run time (5 min) as shown in (Figure 2), so this condition was adopted in subsequent analysis.

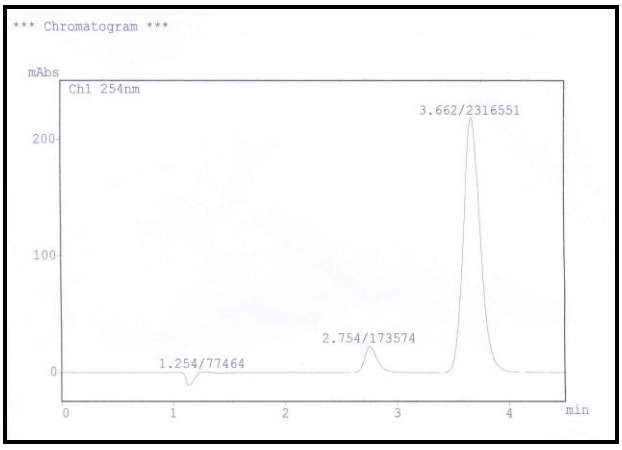


Figure 2: Typical chromatogram of Standrad

Validation Studies

The linearity was determined in the range of 25%-200% for all ingredients. The assay was judged to be linear as the correlation coefficient was greater than 0.998 in all cases, calculated by the least square method. A linear correlation was found between the peak areas and the concentrations in the assayed range. The regression analysis data are presented in Table 1. Chromatogram shown in Figure 3 proves specificity or selectivity of the assayed method, as the chromatograms in samples were found identical with standard chromatogram and no interference peak was observed. Peak purities higher than 98.0% were obtained in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with peaks of interest. The chromatogram obtained with the mixture of the suspension excipient proved that there was no interference between excipient and peak of interest. All the requirements of symmetrical peak were fulfilled, and hence the specificity was confirmed.

Intra-day precision was determined by analyzing Gel sample solutions at different time intervals on the same day. Inter-day precision was determined by analyzing Gel sample solutions on three different days. Gel sample solutions were prepared and analyzed in the similar manner as described in analysis of the gel formulation. Results of intra-day precision and inter-day precision are shown in Table 2.

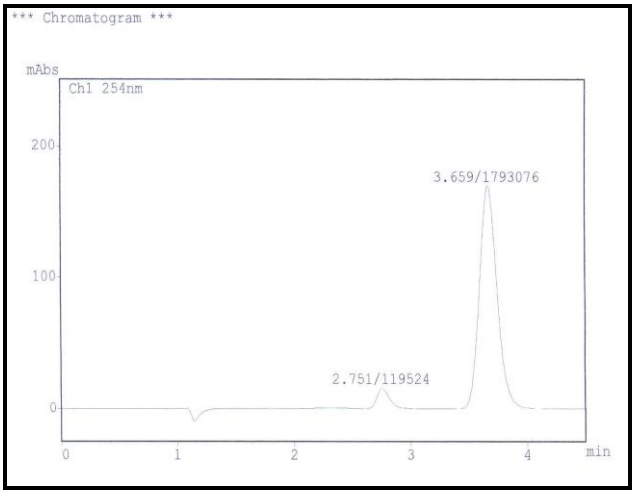


Figure 3: Typical chromatogram of Gel sample

Table 1: Calibration curve data and validation parameters

Parameter	Inference	
	KETO	MP
Linearity range (µg/ml)	13-104	0.55-4.4
Standard Regression equation (y=AX+B) Slope (a)	73363428x+129808	127049x-11423
Correlation coefficient (R^2)	0.9998	0.9997
Limit of detection (LOD) (ng/ml)	2.5	1.25
Limit of quantification (LOQ) (ng.ml)	7.55	3.75

Table 2: Inter-day and intra-day precision and recovery studies

Drugs	Intra - Day Precision Data			Inter - Day Precision Data		
	(%) Mean[a]	S.D.	C.V.	(%) Mean[a]	S.D.	C.V.
KETO	100.02	±0.417	0.415	99.91	±0.311	0.321
MP	99.89	±0.591	0.608	99.15	±1.706	1.717

[a] Average of six determinations

Table 3: Results of recovery studies

Level of recovery	% of gel taken	Amount of drug added, %		Amount of drug recovered, %		% Recovery	
		KETO	MP	KETO	MP	KETO	MP
50%	50.00	50.00	55.00	49.73	54.80	99.47	99.61
	50.00	50.00	55.00	50.20	55.30	100.4	100.5
	50.00	50.00	55.00	48.97	54.90	97.94	99.88
100%	100.0	100.0	110.0	99.92	109.3	99.92	99.33
	100.0	100.0	110.0	100.4	110.3	100.4	100.3
	100.0	100.0	110.0	100.1	109.6	100.1	99.64
150%	150.0	150.0	165.0	149.5	163.2	99.67	98.89
	150.0	150.0	165.0	152.2	165.5	101.5	100.3
	150.0	150.0	165.0	151.5	166.3	100.9	100.8

Table 4: Statistical validation for recovery study

% Mean recovery		Standard deviation		% RSD	
KETO	MP	KETO	MP	KETO	MP
100.03	99.92	±1.001	±0.611	1.001	0.470

Table 5: Results of degradation study

No	Stress condition	Assay of active substance, %	
		KETO	MP
1.	Acid (0.1 mol/l HCl)	99.01	99.15
2.	Alkali (0.1 mol/l NaOH)	98.83	96.49
3.	Oxide (3% H_2O_2)	98.78	95.69
4.	Heat (60ºC)	97.85	99.55

The accuracy was investigated by means of addition of reference standards to a mixture of the suspension excipients. Recovery studies of the drug were carried out for the accuracy parameter at three different concentration levels. A known amount of standards were added into pre-analyzed sample and subjected to the proposed HPLC method. Amount of the drug recovered (%) and recovery percentage were calculated and the results of recovery studies and statistically are shown in Tables 3 and 4.

RESULTS AND DISCUSSION

The proposed method was validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness. In the present work the high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of two compounds in a formulated gel. The two drugs were satisfactorily separated with t_R values of 2.75 min and 3.66 min for Methylparaben and Ketoprofen, respectively. Calibration curves were in the range 13-104 µg/ml and 0.55-4.4 µg/ml for Methylparaben, and Ketoprofen, respectively. Correlation coefficient (r) values were 0.9998, 0.9997 for Ketoprofen and Methylparaben, respectively. A low relative standard deviation (<2%) was found for both precision and robustness study showing that the proposed method was precise and robust. The method had an accuracy of 100.03% and 99.92% for Ketoprofen and Methylparaben, respectively.

The method had the potential to determine these drugs simultaneously in gels without any interference, in accordance with ICH guidelines. Forced degradation of hydrolysis (acidic and alkaline), oxidation and thermal stress were performed as per the conditions suggested in the ICH guideline Q1A (R^2). The drug showed instability in acid and oxide conditions.

CONCLUSION

The proposed HPLC method described in this paper provides a simple, convenient and reproducible approach for the simultaneous identification and quantification of Ketoprofen and Methylparaben in pharmaceutical formulation gels with good separation and resolution. In addition, this method has the potential application to clinical research of drug combination. Analytical results are accurate and precise with good recovery and lowest detection limit values. In short, the developed method is simple, sensitive, easy, and efficient having short chromatographic time and can be used for routine analysis in QC laboratory.

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